HPTLC METHOD FOR THE ASSAY OF TRAMADOL AND PENTAZOCINE FROM MIXTURES

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Abstract

Drug consumption highly increased in recent years and the necessity of therapeutic monitoring of certain medicines (to avoid overdosage or in detoxication treatment of addicts) resulted in the necessity to diversify the opiates analytical methods, mainly in biological matrices (body fluids, tissues, etc.)

The present paper aims to evaluate a high performance thin layer chromatography (HPTLC) method for the assay of tramadol and pentazocine from mixtures in view of further use of the method for the two analgesics assay on biological matrices (blood, urine, tissue). The two drugs were tested using silicagel 60 F_{254} glass plates as stationary phase and two mobile phases, consisting in cyclohexane:toluene:diethylamine (15:3:2) and acetic acid:n-buthanol:water (1:4:1). The densitometric detection was performed in the absorbance (reflectance) mode at 254 nm. The semi-quantitative analysis lead to linear regression curves with correlation coefficients over 0.99. The quantification limits, of 2.03 µg for pentazocine and 0.797 µg for tramadol, allow the quantification of the two substances at toxic levels.

Keywords: tramadol, pentazocine, HPTLC, qualitative analysis, semi quantitative analysis

Rezumat

Creșterea consumului de droguri, precum și necesitatea monitorizării terapeutice a unor medicamente (necesitatea evitării supradozajelor, sau în tratamentele de dezintoxicare a toxicomanilor) a condus la diversificarea metodelor de analiză a opiacelor, cu precădere în matrice de natură biologică (fluide biologice, țesuturi, etc.)

Scopul prezentei lucrări este de a evalua performanțele unei metode HPTLC (high performance thin layer chromatography) de determinare a tramadolului și pentazocinei din amestec în scopul utilizării sale ulterioare la determinarea celor două analgezice din matrice biologice (sânge, urină, țesut, etc.). Cele două medicamente au fost testate utilizând ca fază staționară silicagelul tip 60 F_{254} pe sticlă și două faze mobile: ciclohexan:toluen:diethylamină (15:3:2) și acid acetic:n-butanol:apa bidistilată (1:4:1). Detecția s-a realizat prin densitometrie la 254 nm, în modul de lucru absorbție (reflectanță). Analiza semicantitativă a condus la dreptă de regresie cu coeficienți de corelație mai mari decât 0.99. Limitele de cuantificare evaluate, 2.03 µg pentru pentazocină și 0.797 µg pentru tramadol, permit cuantificarea celor două substanțe la nivele toxice.

Keywords: tramadol, pentazocine, HPTLC, qualitative analysis, semi quantitative analysis
Introduction

Tramadol and pentazocine are synthetic centrally acting opioid analgesics used to treat moderate or severe acute or chronic pain.

The clinical effects of tramadol involve both opioid and non-opioid mechanisms. Tramadol has analgesic activity due to moderate affinity for μ opioid receptors; most of the analgesic effects are attributed to the non-opioid properties (i.e. blocking the reuptake of biogenic amines, norepinephrine and serotonin) [7,8].

Pentazocine has both agonistic actions and weak antagonistic activity on the opioid receptors [5]. The analgesic effects of pentazocine are attributed to agonistic actions on k-opioid receptors.

Currently both tramadol, and pentazocine are frequently reported in the abuse consume by the addicted subjects. There have been reports of pentazocine abuse resulting in moderate dependence (the potential to develop dependence is more pronounced for the injection administration) [2].

Although tramadol is thought to have less potential for abuse compared to other similar medications (i.e. morphine, oxycodone, hydrocodone), it is recommended to avoid tramadol in patients with a history of addiction [3]. It is most likely that tramadol is abused by people with chronic pain or narcotics abusers.

The elaboration of the methods for the identification and quantification of drugs in biological matrices (blood, urine, tissue homogenate), alone or in mixture, at therapeutic and/or toxic concentrations is a major goal in analytical toxicology. Detection and quantification of drugs in biological samples provide specialists (physicians, health care authorities, representatives of law) and an objective tool for the diagnostic of the abuse.

This study aimed to develop a high performance thin layer chromatography (HPTLC) method for the semi quantitative determination of pentazocine and tramadol from mixtures, as exponents of the opioid analgesic class and abuse substances as well.

Materials and methods

Reagents
- Tramadol hydrochloride (T) (donated by LaborMed Pharma) – stock solution of 1 mg/mL in ethanol
- Pentazocine (P) - Fortral® tablets containing 50 mg pentazocine hydrochloride (from KRKA) - stock solution of 1 mg/mL in ethanol
- Pre-coated TLC plates silicagel F254 on glass, 20x20cm, from Merck
- Solvents for the mobile phases: cyclohexane, toluen, diethylamine, acetic acid, n-buthanol – all analytical grade reagents.

**Devices**
- Semiautomatic spotting system Linomat 5 (Camag, Switzerland)
- TLC densitometer Scanner 3 (Camag, Switzerland)
- WinCATS software ver. 1.4.4.

**Procedure**

On the standard plates, different amounts of stock solutions were spotted under nitrogen, in lines of 10 mm length, as summarised in Table I.

**Table I.**

<table>
<thead>
<tr>
<th>Track No.</th>
<th>Substance</th>
<th>Amount (µL)</th>
<th>1-5</th>
<th>6-8</th>
<th>9-14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tramadol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixture T:P (1:1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pentazocine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Plates were developed in a vertical chamber, previously saturated with the mobile phase vapors, in a dark place, using cyclohexane:toluene:diethylamine (15:3:2) and acetic acid:n-buthanol:water (1:4:1) as mobile phases [6, 4].

The examination of the plates was performed by densitometry, using UV light at $\lambda=254$ nm, in the absorbance (reflectance) mode; the retention factor ($R_f$) value was automatically computed for each track on the basis of the maximum peak for each spot. The mean $R_f$ value and standard deviations were computed for all the corresponding spots on all tracks.

For the semi-quantitative evaluation of the two substances, the quantitative analysis facility of the software WinCATS ver. 1.4.4 was used.

**Results and discussion**

The chromatoplates obtained for the two solvent systems used showed that only the mobile phase acetic acid:n-buthanol:water (1:4:1) can be used for the qualitative assay of the two substances (densitogram presented in Figure 1).

The $R_f$ values obtained for the two substances, as a mean of the $R_f$ values computed on the peaks for the corresponding tracks were 0.54 ±0.01 for tramadol and 0.82 ±0.01 for pentazocine.

The UV spectra of the corresponding spots of pentazocine and tramadol (Figure 2) show that the two substances have not only different $R_f$ values, but also different reflection spectra, whose peaks are at 279 and 283 nm for tramadol and pentazocine, respectively, corresponding to the values obtained for absorption UV spectrum of each substance (not shown).
The two substances are well separated when using acetic acid:n-buthanol:water (1:4:1) as a mobile phase, therefore that was the choice for the semi-quantitative assay of the two substances from mixture.

Figure 1
3-D representation of the densitogram for tracks 1-14 of the plates developed with acetic acid:n-buthanol:water (1:4:1)

Figure 2
UV absorption (reflectance mode) of the corresponding spots for pentazocine
The semi-quantitative assay of the two substances was performed by using the quantitative analysis facility of the software. For the assay, the peak area was taken into account, and the representation of the linear regression obtained for each substance is depicted in Figure 3. The parameters obtained for each calibration curve are given in Table II.

![Calibration curves for pentazocine (a) and tramadol (b)](image-url)
Table II. Parameters obtained for the regression curves

<table>
<thead>
<tr>
<th>Substance</th>
<th>$Y = A \times x + B$</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentazocine</td>
<td>217.3 1612</td>
<td>0.99337</td>
</tr>
<tr>
<td>Tramadol</td>
<td>322.4 740.5</td>
<td>0.99525</td>
</tr>
</tbody>
</table>

On the basis of the regression curves, using the ICH guidelines [1] for the validation of the analytical procedures, the computed detection (DL) and quantification limits (QL) of the two compounds by the method described above were as follows: pentazocine – DL=0.670 µg, QL=2.030 µg; tramadol – DL=0.263 µg, QL=0.797 µg.

Conclusions

We developed a high performance thin-layer chromatographic method for the assay of pentazocine and tramadol from mixture. The substances were spotted in lines under nitrogen flow on silicagel 60 F$_{254}$ Merck precoated plates, the mobile phase identified as giving optimum performance being the mixture acetic acid: n-butanol : water (1:4:1). The plates were analysed by densitometry at 254 nm, the obtained $R_f$ for the two substances was 0.54 ±0.01 for tramadol and 0.82 ±0.01 for pentazocine, thus the two substances can be well separated. The semi-quantitative assay lead to a linear regression when using both the peak maximum and the peak area, with correlation coefficients over 0.99. The quantification limits, of 2.03 µg for pentazocine and 0.797 µg for tramadol, allow the quantification of the two substances at toxic levels (greater than 1 µg/mL for pentazocine and 0.2 µg/mL for tramadol).

References

1. *** Validation on Analytical Procedures: Text and Methodology – ICH Q2 (R1) – International Conference on Harmonisation, Geneva, Switzerland, 2005

*Manuscript received: November 11th 2010*